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The new therapeutic horizons for acute lymphoblastic leukemia

Literature review

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Resumo

A terapêutica da leucemia linfoblástica aguda (LLA) em idade pediátrica é um exemplo de sucesso em oncologia e está associada a altas taxas de cura. Porém, há ainda muitas crianças e adultos que recidivam ou que apresentam doença primariamente refratária, sendo que a maioria tem doença considerada resistente. A aplicação de "regimes pediátricos" de quimioterapia a adultos que tolerem, leva à obtenção de melhores resultados nesta população. Além do transplante alogénico de células estaminais hematopoiéticas, a investigação de novas terapêuticas para a LLA dirigidas a alvos moleculares específicos é considerável e novos fármacos surgem. Destacam-se: a utilização de inibidores tirosina cinase na LLA Philadelphia+ bem como o seu potencial num subtipo recentemente identificado da doença – LLA Philadelphia-like; e a imunoterapia que se afirma também neste contexto através da utilização de anticorpos monoclonais como o blinatomumab (recentemente aprovado pela EMA e FDA) bem como a infusão de células T geneticamente modificadas – CAR T cells. O melhor conhecimento da biologia da LLA de fenótipo T, para a qual as terapêuticas supramencionadas não se revelam muito promissoras, é essencial - há avanços, apesar de desproporcionais. Novas opções terapêuticas que atualmente são utilizadas apenas na terapêutica de resgate poderão ser implementadas nos regimes iniciais num curto prazo.

Abstract

Therapy for acute lymphoblastic leukemia (ALL) in pediatric age is an example of success in oncology and is associated with high cure rates. However, there are still many children and adults who relapse or have primarily refractory disease, most of them have resistant disease. The application of "pediatric inspired" chemotherapy regimens to adults who tolerate, improve outcomes in this population. Beyond allogenic hematopoietic stem cell transplantation, investigation of new therapies for ALL directed at specific molecular targets is considerable and new pharmacologic agents are arising. These include: the use of tyrosine kinase inhibitors in Philadelphia chromosome positive ALL as well as its potential use in a recently identified subtype of the disease – Philadelphia-like ALL; and immunotherapy that appears in this context through the use of monoclonal antibodies, such as blinatumomab (recently approved by EMA and FDA), as well as the infusion of genetically modified T cells – CAR T cells. A better knowledge of T phenotype ALL biology, for which the aforementioned therapies do not reveal as promising, is essential – there is progress although disproportionate. New therapeutic options that, at present, are used only as rescue therapy may be implemented in frontline regimens in a near future.

1. Introduction

1.1. Perspective of ALL with standard therapy and its outcomes

ALL is among the few oncologic diseases that do not spare age groups. 60% of cases are diagnosed in patients under the age of 20 and 11% in patients older than 65 [1,2]. This makes the approach to ALL a complex one, once patient and leukemic factors must be considered when planning therapy [1].

Chemotherapy regimens for the treatment of pediatric ALL are considered an example of success in oncologic therapy[1,3]. The optimization of therapeutic regimens provided cure rates as high as 90% for patients who once had a dismal prognosis [1,4,5]. The improvements in survival are due primarily to the decrease in relapse rates, with weak improvements during the last 20 years for the children who still relapse.

For the adults the same success was not found using similar approaches. These regimens result in complete remission (CR) rates for about 80 to 90% but only 40 to 50% for cure [1,6,7]. The addition of agents directed at specific targets improved survival and the cure rates in adults [1,7,8].

Relapsed ALL continues particularly challenging in all age groups making it one of the main causes of cancer related death in children worldwide and having an even worse prognosis in adults.

The majority of children who relapse is going to reach a second complete remission (CR2) in contrast to the adult population that in the best scenario do it in less than 50% of cases. Even when a second complete remission is obtained, the response is not sustainable in most cases. For each subsequent relapse, achieving a new remission becomes increasingly difficult and the survival becomes extraordinarily low.

Refractory ALL is also challenging with a survival rate close to 30%. For those who do not achieve a remission the options are limited.

1.2. New horizons for ALL therapy

The purpose of this review is to integrate the major advances concerned with ALL therapy that are being reported in the literature. Subjects related to standard chemotherapy regimens; bone marrow transplantation and general support to the oncologic patient will not be discussed in detail here.

In the present review subjects will be presented as follows:

- A major modification in standard chemotherapy regimens – the application of pediatric "inspired" regimens to the adult population.
- Targeted therapies to Philadelphia Chromossome + ALL and the potential to adopt a similar strategy in a recently described entity of the disease – Philadelphia like ALL (Ph-like ALL).
- Immunotherapy as a treatment for ALL - Probably the most highlighted subject in virtue of its recent promising results with different monoclonal antibodies and the use of CAR T cells.
- T phenotype ALL will be discussed afterwards once the most powerful directed therapies being developed at present are not used to treat this subtype of the disease however progress is also being made.

2. Application of pediatric intensive chemotherapy regimens for adolescents and young adults (AYA) with ALL

There is not a universally accepted definition of age subgroups in ALL however it can be considered that classic pediatric ALL patients range from 0 to 14 years, adolescents from 15 to 19, young adults from 20 to 39, adults from 40 to 60 and older adults and elderly patients include those beyond the age of 65.

Several biologic factors are present in less young patients that confer them a poorer prognostic, such as differences in cytogenetics, molecular genetics, immunophenotype and response and tolerability to intensive chemotherapy regimens.

Regimens applied to adults typically consist in the use of intensive mielossupressive agents including daunorubicin, cytarabine, cyclophosphamide as well as allogenic hematopoietic stem cell transplant when complete remission is achieved (16,17,18).

By contrast, pediatric regimens focus on the Berlin-Frankfurt-Munster (BFM) ALL therapy framework or on the Dana Farber Cancer Institute (DFCI) ALL consortium protocols: glucocorticoids, vincristine, asparaginase, CNS prophylaxis and prolonged maintenance therapy. Asparaginase related toxicity is one of the major problems when pediatric regimens are applied to adults **(9,13,19,20).**

The success achieved in the treatment of pediatric ALL did not apply to adults (9,10). In the last decades, the treatment of adults with ALL resulted in survival rates of around 40% (11,12). However, multiple studies have demonstrated that AYA treated with pediatric regimens of intensive chemotherapy achieve better responses than when treated with standard adult intensive chemotherapy regimens.

This was first demonstrated in a retrospective comparative study with 321 AYA that were treated in various clinical trials either by the Children's Cancer Group (CCG) or by the Cancer and Leukaemia Group B (CALBG) from 1988 to 2001 (13).

CR rates among pediatric and adult cohorts were the same, however AYA patients treated by the CCG achieved a global survival at 7 years of 67% in contrast to a global survival of 47% achieved by AYA patients treated by the CALGB.

Similar studies in other countries have shown similar outcomes (14,15).

Based on these retrospective comparisons, several prospective studies using "pediatric inspired" and "unmodified pediatric protocols" in AYA patients in different countries have shown favourable outcomes with uniformity. These studies included both standard and high risk ALL patients. The biggest prospective study in this subject is the ongoing US intergroup study, C10403, and aims to demonstrate that adult cancer cooperative groups can deliver a "true pediatric'' regimen to AYA patients achieving similar outcomes.

Explaining the differences in outcome between pediatric and adult protocols are several factors:

- Differences in protocol design and treatment intensity, with pediatric protocols including more nonmielossupressive agents with activity on lymphoblasts: asparaginase, vincristine and glucocorticoids.
- Central nervous system (CNS) prophylaxis is administered earlier, with greater frequency and for more prolonged periods in pediatric trials.
- Possibly, a more accurate administration of therapy in pediatric institutions and a better compliance of adolescent patients treated in a pediatric facility.

The age limit for the administration of pediatric intensive regimens is not well defined. Some studies used these regimens in patients up to 50 to 60 years old. Therapy related mortality and toxicity increase in older patients (14). Differences in the metabolism of chemotherapeutic agents, depleted marrow reserve and greater extramedullary toxicity increase the frequency of life threatening infections, organ failure, treatment delays and dose reductions in planned therapy in these patients.

Based on these studies, current recommendations suggest treating AYA patients with pediatric regimens since diagnosis with the purpose of achieving remission and potential cure (21).

3. Philadelphia chromosome positive (Ph+) ALL and Philadelphia chromosome-like (Ph-like) ALL – the role of tyrosine kinase inhibitors

3.1. Philadelphia Chromossome positive (Ph+) ALL

Philadelphia chromosome arises from a translocation of the ABL gene on chromosome 9 to the BCR gene on chromosome 22 giving rise to a chimeric BCR-ABL fusion gene. A Bcr-Abl protein results, being a constitutively active tyrosine kinase, central to the pathogenesis of ALL. A tyrosine kinase is an enzyme responsible for the activation of proteins in signal transduction cascades by phosphorylation. This aberrant Bcr-Abl tyrosine kinase leads to leukemogenesis altering signalling pathways that lead to increased cell proliferation, survival and self-renewal.

Philadelphia chromosome is present in approximately 20 to 30% of adults with ALL but in only 2 to 3% of children [22,23]. It is the most common chromosomal abnormality in adult ALL population.

Recently, greater interest has arisen in the underlying biology of this specific leukemia associated with the fact that more mature information about its treatment has been accumulated [24].

The bad prognosis of this relatively rare leukemia has led to the adoption of therapeutic strategies like the allogenic hematopoietic stem cell transplant (aHSCT) and tyrosine kinase inhibitors (TKIs), despite the scarcity of randomized clinical trials [24]. In adults aHSCT in first remission remains the only proven curative strategy for transplant eligible patients. The introduction of TKIs has improved the depth and duration of CR allowing more patients to proceed to transplant.

The first Bcr-Abl TKI was imatinib and it was approved for the treatment of chronic myeloid leukemia (CML) showing high efficacy; subsequently, second generation TKIs were developed with the purpose of overcoming resistance and intolerance to imatinib. Dasatinib, nilotinib, bosutinib and ponatinib are currently available second-generation Bcr-Abl TKIs.

Early detection of *t(9,22)* is now a vital component of the approach to ALL. Bone marrow samples should be sent for cytogenetic and molecular study accompanied by a specific request for detection of Philadelphia chromosome/ BCR-ABL [24].

In the past, chemotherapy applied to Ph+ ALL was identical to that applied to Ph- ALL, however patients with Ph + ALL achieved complete remissions less frequently, with CR rates of 60 to 85% [25], and relatively short durations of remission of about 9 months. It was assumed then, that chemotherapy applied to these patients should consist in the most intensive regimens available.

Once the outcomes with standard chemotherapy were discouraging, TKIs were adopted globally in Ph+ ALL treatment protocols after showing promising outcomes in initial studies that demonstrated activity in relapsed disease [26,27].

Imatinib is the most used and most extensively studied tyrosine kinase inhibitor in the context of ALL. It has been combined with chemotherapy in different schedules and timings however there has never been a randomized controlled trial so its benefits are evaluated by reference to historic control patients [24]. Several retrospective and prospective studies of the use of imatinib in Ph+ ALL are now published and they all have as a prominent finding a high CR rate of approximately 95% [28,29,30]. When used in patients with *de novo* Ph+ ALL it improves complete response (hematologic, cytogenetic and molecular), prolongs time to relapse, improves transplant eligibility and leukemia free survival.

There are theoretical advantges in the potential initial use of second generation TKI's like increased potency (dasatinib, nilotinib, bosutinib and ponatinib), broader spectrum of activity in kinase inhibition (dasatinib and ponatinib) and better penetration in the CNS potentially reducing local relapse (dasatinib).

Results from studies of dasatinib are beginning to be available [24]. In an Italian study, 100% of patients with de novo Ph+ ALL who were treated with dasatinib in combination with steroids (but without chemotherapy) achieved CR within 1 month of starting the treatment [31].

Ponatinib has been reported to be effective in CML patients whith the T315I mutation which confers resistance to imatinib, so it can be considered in ALL if there is evidence of such mutation.

No studies have examined the optimal duration of treatment with TKIs during induction, consolidation and maintenance. Comparative clinical data and literature is still insufficient regarding comparisons between TKIs.

3.2. Philadelphia chromosome-like (Ph-like) ALL

Ph-like ALL, a recently described entity, is characterised by a similar gene expression profile to Ph+ ALL; alterations in lymphoid transcription factor genes; and a bad prognosis [32,33]. Patients with Ph-like ALL do not have the BCR-ABL1 fusion gene, instead, a diverse range of genetic alterations that deregulate cytokine receptor and tyrosine kinase signalling has been observed.

Ph-like ALL increases in frequency from 10% among children with standard risk ALL to 27% among young adults and is associated with a poorer outcome.

The frequency and spectrum of genetic alterations in Ph-like ALL and the response to tyrosine kinase inhibitors is yet undefined.

About 90% of patients with Ph-like ALL have kinase activating alterations [34]. There are distinct subgroups of kinase and cytokine receptor genes alterations; The frequency of these subgroups also varies with age:

- Rearrangements involving ABL-class tyrosine kinase genes (ABL1, ABL2, CSF1R, PDGFRB) predicted to respond to ABL1 inhibitors (12,6% of cases);
- Rearrangements involving CRLF2 (49,7% of cases) predicted to respond to JAK2 inhibitors in many cases;
- Rearrangements of EPOR (3,9% of cases) or JAK2 (7,4% of cases) predicted to respond to JAK 2 inhibitors;
- Genetic alterations of IL7R, FLT3, SH2B3, JAK1, JAK3, TYK2, IL2RB
- RAS pathway mutations (4,3% of cases)
- Uncommon fusions involving NTRK3 or DGKH (0,9% of cases)

The expression of fusion products involving ABL1, ABL2, CSF1R and PDGFRB resulted in cytokine-independent proliferation and activation of phosphorylated STAT5 [34].

Cell lineages and human leukemic cells that expressed fusions with ABL1, ABL2, CSF1R and PDGFRB were sensitive in vitro to dasatinib (ABL inhibitor), the

rearrangements with EPOR and JAK2 were sensitive to ruxolitinib (JAK inhibitor) and the fusion ETV6-NTRK3 to crizotinib (ALK inhibitor) (35,36,37).

Direct targeting of RAS is challenging but targeting of signalling pathways downstream of this protein may be considered.

The frequency of Ph-like ALL is higher than 25% among young adults with ALL. Once BCR-ABL1 + ALL represents more than 20% of pre B ALL cases in this age group, about half of young adults with pre B ALL are candidates for tyrosine kinase inhibitory therapy.

Opportunities to match genomic discoveries with the management of ALL are becoming available, from initial diagnosis and risk stratification to the delivery of targeted therapy. Sequencing will be increasingly used for molecular diagnosis because many genetic alterations are undetectable using our current tests – Ph-like ALL is a notable example (34).

Clinical trials with tyrosine kinase inhibitors in this population are warranted.

4. Immunotherapy in ALL

4.1. Rational of Immunotherapy in ALL

Malignant cells are continuously eliminated by apoptosis and by the immune system yet cancers escape these mechanisms. So that immune mediated clearance of leukemic cells becomes possible, immune tolerance must be overcome. This is the basis for the graft-versus-leukemia effect that contributes in part for the efficacy of allogenic hematopoietic stem cell transplant and is also the rational for the donor lymphocyte infusion in leukemia (38). Although donor lymphocyte infusion has revealed to be less effective in ALL, graft versus leukaemia effect is recognized.

A recent clinical trial from the Children Oncology Group demonstrated that the relapse rate in children who developed graft-versus-host disease after allogenic hematopoietic stem cell transplant was significantly lower than in children who did not develop it (39). However the graft versus leukaemia effect carries a significant risk of graft versus host disease.

4.2. Therapy with monoclonal antibodies

Leukemic blasts in ALL express several surface antigens susceptible of being targeted by directed therapies including CD19, CD20, CD22 and CD52. Monoclonal antibodies are able to target these antigens selectively, minimizing off-target toxicity. Monoclonal antibodies exert their function through various mechanisms that include direct cytotoxicity, complement-dependent cytotoxicity and apoptosis induction. If the target internalizes upon binding with the antibody then potent cytotoxines can be conjugated producing an additional mechanism (1).

When added to first-line chemotherapy, rituximab (anti-CD20), increased Burkitt leukemia cure rates in adults from 40% to 80% and pre B-ALL cure rates from 35 to 50% (8). Inotuzumab ozogamicin (anti-CD22 linked to calicheamicin) originated complete remission rates of 55% and median survival of 6 to 7 months when given to patients with relapsed/ refractory ALL (1,40,41). Blinatumomab – a biallelic monoclonal antibody to T cells CD3-CD19 has also resulted in global responses of 40 to 50% and a median survival of 6,5 months in a similar population (1,42). Other promising monoclonal antibodies against CD20 (ofatumumab and obinutuzumab) or CD19 and linked to different cytotoxins or immunotoxins are in development.

4.2.1. CD20 directed therapy

CD20 surface antigen is observed in 30 to 50% of B cell precursor lymphoblasts (1,43). Rituximab is a chimeric monoclonal antibody originally developed and approved for the treatment of non-Hodgkin lymphoma. Several studies have reported that the addition of rituximab to chemotherapy improved cure rates of Burkitt ALL (8). Two studies have shown the same for pre-B ALL. Rituximab is preconized in addition to

Hyper-CVAD in patients diagnosed with Ph – ALL, CD20+. The majority of protocols restricts its use to patients whose blasts express CD20 in more than 20%. There is an on-going study to determine the safety of intraventricular rituximab in patients with relapsed ALL in the CNS (44). This antibody is generally well tolerated and the majority of its adverse effects are related to the infusion.

Ofatumumab is a second-generation anti-CD20 monoclonal antibody that binds to a different place than rituximab being more potent (1,45). In chronic lymphocytic leukemia this antibody has demonstrated significant activity after previous exposure to rituximab (1,46). In a phase 2 study in pre-B ALL, the combination of HCVAD with ofatumumab was very effective. 25 patients with pre-B ALL *de novo* were treated. CR rates and minimal residual disease rates were both negative in 96%. With a median follow-up of 14 months, disease free survival and global survival rate were 94 and 92%, respectively (47).

Obinutuzumab is a new anti-CD20 antibody that is superior to the latter in the induction of cellular death. Investigation in CD20+ ALL patients is warranted after the promising results that it showed in CLL in combination with clorambucil (1,48).

4.2.2. CD19 directed therapy

CD19 is expressed in B cells since their early stages of differentiation. After the antibody binds to this antigen, it internalizes becoming a potential target of immunoconjugated therapies (1,49).

Blinatumomab is a bispecific antibody that also binds to T cells. It contains both the variable domains of an anti-CD19 and anti-CD3 antibodies linked by a non-immunogenic linker. When it links to CD19, an activation of cytotoxic T cells inducing cellular death by the perforin system occurs(1,50).

The continuous administration during several weeks instead of an intermittent one, 2 to 3 times per week, increased significantly its activity while minimizing adverse effects (50).

The activity of this antibody was already studied in minimal residual disease and in relapsing/ refractory disease (52,53).

The first study of blinatumomab using continuous infusion evaluated its potential in the eradication of minimal residual disease (1,51). Minimal residual disease in ALL is associated almost always with systemic relapse and bad prognosis. A dose of 15ug/m2 was administrated daily as a continuous infusion during 28 days, every 6 weeks. After completing one cycle, patients could receive more 3 cycles of additional consolidation or proceed to allogenic stem cell transplant if a compatible donor was available. Minimal residual disease conversion was observed after completing the first cycle in 16 of 20 evaluated patients (80%). In a longer follow up (median of 33 months) 12 of the 20 patients continued in complete remission, relapse-free survival at 3 years was 60%. 9 patients proceeded to transplant but, surprisingly, with outcomes similar to the non-transplant group. The majority of relapses occurred in the first 7 months after therapy.

A few hours after the beginning of the infusion, a rapid redistribution of T cells was observed – after a rapid reduction, T cells recovered immediately to pre-treatment levels and expanded above the basal level during the rest of the course of the first infusion. This pattern was observed in 8 of 17 patients evaluated. Although the increase was due to the subpopulations CD4+ and CD8+, memory T cells was the predominant subpopulation.

Blinatumomab was subsequently studied in patients with relapsed ALL (52,53). The results of this trial were recently updated. 3 dose schedules were explored using the latter scheme. The global response rate with two cycles of therapy was 69%. The median survival was 9,8 months. The final dosage selected for future trials was 5ug/m2 during the first week followed by 15ug/m2 for the next 3 weeks.

The adverse effects more commonly observed after administration include fever, chills and hipogammaglobulinemia. Tremor, headaches and mental status alterations were also reported. Fever, chills and constitutional symptoms are provoked by a cytokine releasing syndrome that occurs early after therapy and that is reduced with corticosteroids. This syndrome will be discussed later in this article.

There are currently other therapies with immunoconjugates directed at CD19 being developed. SAR3419 is an antibody directed at CD19 conjugated with a maitansinoid compound – an anti-mitotic agent that binds to tubulin (similar to vincristine). These compounds are much more powerful than vinka alkaloids but their toxicity profile has led to their abandonment in conventional chemotherapy. This immunoconjugate has shown efficacy in pre-clinical models. As a limiting factor there is turve vision due to cornea epithelial alterations (1,54,55). SGN-DC19A is another immunoconjugate with the antimicrotubule agent Auristatin F (MMAF). Upon binding to CD19, the compound internalizes delivering MMAF that binds to tubulin inducing G2/M arrest and apoptosis (56). Phase 1 studies are being performed to determine dosage. The adverse effects consist in nausea, fever, chills and headache.

4.2.3. CD22 directed therapy

CD22 is expressed in the lymphoblast in more than 90% of ALL patients (43). After binding to an antibody, the complex is rapidly internalized which makes it an attractive target for antibodies conjugated with cytotoxic agents (57).

Epratuzumab is a non conjugated antibody that has already been studied in pediatric relapsed ALL; with this antibody used alone, the majority of patients had stable disease and only one of 15 treated patients had a partial response. Despite showing modest activity the best therapies directed at CD22 are probably those that use immunoconjugates (58).

Inotuzumab ozogamicin is the more developed anti-CD22 immunoconjugate. The antibody is linked to calicheamicin – a cytotoxin that induces gaps in the double strand of DNA (59). Reversible thrombocytopenia is a frequent adverse reaction (60). Hepatic function abnormalities were also observed as well as the development of veno-oclusive disease in patients that followed to transplant. In relapsed/ refractory ALL this immunoconjugate has shown very promising results which motivated its further study in combination with low intensity HCVAD in older patients (ages between 60 and 79). All the patients with cytogenetic anomalies achieved a complete cytogenetic response and all patients that had a response also had negative status for

minimal residual disease. The survival at one year was superior to previous results obtained with HCVAD+rituximab in a similar population (61).

Moxetumomab pasudotox is an immunotoxin constituted by a variable fragment derived from an anti-CD22 monoclonal antibody fused to pseudomonas aeruginosa exotoxin A. In a phase 1 study, 21 children and adolescents with relapsed/ refractory ALL were treated with this immunotoxin. From the 17 evaluable patients, 24% achieved CR, 6% obtained a partial response and 47% achieved hematologic improvements for a global activity rate of 70% (62). Trials with this antibody administered in a higher frequency and dosage are on-going.

4.2.4. CD52 directed therapy

For the patients with T phenotype ALL, therapeutic options with antibodies are very limited. Alentuzumab is a humanized monoclonal antibody against CD52. This antigen is expressed in 36 to 66% of leukemias, including B ALL; T ALL and AML. However the outcomes obtained with this antibody were not promising adding to its profound adverse effects (63).

4.3. Therapy with CAR-T cells

Therapy with CAR (chimeric antigen receptor) - T cells is an immunotherapy that has emerged as a powerful weapon and has been showing promising responses. In this therapy, T cells are removed from the patient, reprogrammed in laboratory to identify and eliminate malignant cells through tumor-specific antigen recognition, and reintroduced (39).

The T cell begins to show in its membrane a synthetic chimeric receptor for a specific antigen becoming reactive against all cells that express it. Contrarily to T cell receptor (TCR), CAR can link to structures in an MHC independent fashion (64,65).

The receptors are called chimeric because they are made of parts derived from different sources. They are constituted by an ectodomain that contains the signal peptide, the antigen-recognizing region and a spacer; a trans membrane domain; and an endodomain.

The concept was first described 25 years ago. The first design made the linkage of a single chain variable fragment (scFv) derived from an antibody, to the intracellular signalling domain CD3 ζ of TCR, through a trans membrane domain and a spacer. This fundamental design has led to the designation of the first generation CARs (65).

The recruited intracellular signalling domain CD3ζ activates and induces T cell proliferation, however it can lead to anergy. Once it was necessary to optimize T cell proliferation and persistence, both in vitro and in vivo, modifications were made that led to the second and third generation CARS. Second generation CARs incorporate an additional domain – either CD28 or 4-1BB to provide a co-stimulatory signal. Third generation CARS incorporate two additional domains – a combination among CD27, CD28, 4-1BB, ICOS or OX40. It is not known which is the best co-stimulatory combination – similar anti-tumour effects were observed in vitro with second generation CARS with CD28 and with 4-1BB (67), however, pre-clinical studies in vivo suggest that the latter are associated with greater proliferation and persistence of T cells (68).

The spacer and trans membrane domain are probably the least studied subjects and they can have important contributions in the interaction with the antigen, in the formation of the immunologic synapse and in the association of CARs to other proteins necessary to transduce a robust signal activation (39).

There are currently available several technologies of genetic transference that can be used to modify T cells, each of which, with its advantages and disadvantages in their price, safety and level of expression – since viral vectors as y-retrovirus and lentivirus that result in a permanent genome modification to RNA based methods that only lead to a transitory gene expression. Viral vectors have the advantage of long lasting expression, on the other hand they lead to permanent on-target toxicity and risk of transformation if the genic insertion results in an oncogene deregulation, the latter is only a theoretical risk (39,65).

An important variable that influences the proliferative capacity and T cell persistence is the ex vivo culture system used in the production of CAR-T cells (66). Several

systems were developed that use antibodies and/or artificial APC with cytokine support to stimulate the T cells. Their differences influence the final composition in effector, naive and memory T cells. While the first are able to mediate a potent cytotoxicity they have a reduced proliferative capacity because they are terminal differentiated.

Relatively to the ideal target of CAR-T cells in ALL this would be tumour-specific, expressed ubiquitously in all lymphoblasts and not expressed in normal cells. This antigen is difficult to find but there are viable alternatives (39). The surface protein CD19 is expressed during the development of B cell lineage and is expressed in almost all of B cell cancers including ALL, CLL and many non-Hodgkin lymphomas. Its level of expression as well as its specificity for B cell has made it an attractive target for CAR-T cells. Other B cell specific molecules as CD22 are also promising and are under current investigation (39).

The identification of targets to phenotype T ALL is a particular challenge once lymphoblasts express the same antigen as normal T cells. While B cell aplasia is treatable and tolerable, T cell aplasia is not. Although some specific subgroups of T cell leukemias express aberrantly abnormal fusion proteins there is no universal target.

At present, all publications about CAR T cells usage in hematologic malignancies are in B cell cancers having as a target CD19 or CD20. The other one refers to acute myeloid leukaemia having as a target the Lewis-Y antigen (65). Each group has designed different protocols and they vary in the design of CARS, its expression in T cells, cellular culture conditions, lymphodepletion strategy used and timing of the infusion of CAR-T cells in relation to standard therapy (65).

It is not surprising that CAR-T cells directed at hematologic malignancies have been the first to be tested, given the extent of knowledge about surface antigens expressed in these cancers, the relative simplicity in obtaining tumour samples and the natural preference of T cells in homing to hematologic organs as the blood, bone marrow and lymphoid nodes.

The clinical trials in CLL and ALL demonstrated high activity and very good clinical responses. Initial reports included a low number of patients but were notable in the remissions induced in patients with relapsed/ refractory CLL and in patients with highly refractory ALL that were considered incurable (65).

CR rates as high as 90% were observed in children and adults with relapsed/ refractory ALL treated with CAR-T cells directed against CD19 (39).

The first reports were expanded to determine the complete remission rate and at present 3 groups using different models of CARs have published their results. The efficacy is even better than expected with CR rates of about 70 to 90%.

- A CR rate of 90% was obtained in a cohort of 30 patients (children and adults) with relapsed/ refractory ALL treated in the Children's Hospital of Philadelphia (CHOP) and University of Pennsylvania (70).
- A CR rate of 88% was obtained in a cohort of 16 adult patients with relapsed ALL treated in the Memorial Sloan-Kettering Cancer Center (MSKCC) (69).
- A CR rate of 70% was obtained in a cohort of 20 children and young adults with ALL in an intent-to-treat analysis of the National Cancer Institute (71).

The three studies included patients with previous history of allogenic hematopoietic stem cell transplant not having had graft versus host disease. The initial response is comparable in all studies, however CARs persistence and long term outcomes can vary and distinguish CAR designs.

Distinct design of CARs in various studies produced similar results. Durable remissions were observed in patients not subjected to additional therapy. Data has suggested that CAR-T cells design is associated with cell persistence and remission duration, however more studies are necessary with a more regular follow-up.

The most common and potentially dangerous adverse reaction associated with this therapy is cytokine releasing syndrome. There is a correlation between the development of this syndrome and the response to therapy. Patients who do not develop it seem to have less benefit (39).

Similarly to other T cell activating therapies, including therapy with blinatumomab, the severity of this syndrome is related to the tumour burden at the time of the infusion.

Cytokine releasing syndrome is a systemic inflammatory process derived from the exponential proliferation of T cells with resultant marked elevation in cytokine levels. Symptoms vary from mild flu-like symptoms to shock and multiorgan insufficiency. The cytokine elevation profile resembles that seen in macrophage activation syndrome/ hemophagocytic lymphohistiocitosis with marked elevations of sIL2Ra, IL-6, IL-10 and IFN-y. Patients develop similar clinical and laboratory features including marked hyperferritinemia, hepato/splenomegaly and hipofibrinogenimia (39).

A better knowledge of this syndrome is needed to predict which cytokines are necessary for therapeutic efficacy, and which are not and that can become potential candidates for pharmacologic targeting.

The high levels of IFN-y liberated by cytotoxic T cells may be important to therapeutic efficacy. The same is true for the high levels of sIL2-Ra. IL-10 is a negative regulator of macrophage function so that its inhibition could actually worsen the syndrome. IL-6 is a pro-inflammatory cytokine that may be a good target for inhibition when treating this syndrome (39).

IL-6 antagonists have already been used in cytokine releasing syndrome. Tocilizumab was given to ten patients and a frank improvement was observed without alterations in therapy efficacy. The majority of patients had a rapid improvement and complete resolution of the syndrome. This agent is well tolerated with rare toxicities that include hepatic inflammation and cytopenias, but they were reported only in chronic use. There are other IL-6 antagonists like siltuximab, sarilumab and olokizumab (69).

Corticosteroids are used frequently in this syndrome when it is triggered by blinatumomab, however there are concerns about its potential neutralizing effects in CAR-T cells function when administered in this scenario.

Tumour burden in the moment of the infusion is the best predictor of severity of this syndrome. CRP was proposed as a marker of severity once its elevation is proportional to the severity of the syndrome, however its role as predictive biomarker is currently under investigation. In future, cytokine levels monitoring might be used to determine the need for therapy with cytokine blocking agents like tocilizumab (39).

After T cell activating therapies, severe neurologic toxicities can occur. Global encephalopathy is the most common adverse reaction however seizures are also possible. The encephalopathy is generally mild and self-limited. CT scans and MRI as well as lumbar puncture have not revealed a specific etiology for this syndrome. CAR-T cells have been found in the cerebrospinal fluid in the majority of patients even those who do not develop neurologic toxicity. Tocilizumab administration does not appear to decrease the incidence of these adverse reactions (39,69)

Chronic B cell aplasia and resultant hypogammaglobulinemia is an on-target adverse reaction that is expected in this therapy as it eliminates normal mature B lymphocytes as well as pre-B cells. As CAR-T cells persistence increases, the greater is this toxicity – in fact it functions as a good pharmacodynamic biomarker. Despite the fact that iv administration of immunoglobulin decreases most infectious complications, a longer follow-up is necessary to evaluate late consequences of B cell aplasia (39).

5. Advances in the treatment of T-ALL

T-ALL accounts for 15% of childhood cases and 25% of adult cases of ALL. Current chemotherapy regimens cure about 85% of children with T-ALL but only 50% of adults. T-ALL treatment evolved since the use of standard non-Hodgkin lymphoma regimens to standard ALL regimens. The application of pediatric intensive chemotherapy regimens to AYA has significantly improved prognosis, as previously mentioned (72).

An important therapeutic agent in T-ALL treatment is nelarabine. This agent has already a well established role in relapsed/ refractory ALL achieving CR rates of 31%, global response rates of 41% and 1-year survival of 28%. It has been combined with first line chemotherapy in both adult and pediatric patients. In adults it has been

combined with hyper-CVAD as initial treatment. Neurotoxicity is the most important side effect (72).

The development of new agents for T-ALL therapy did not follow that for B-ALL. Molecular studies are improving our knowledge on T-ALL pathogeny and the discovery of NOTCH1 and FBXW7 activating mutations was very relevant.

Activating mutations in the gene encoding NOTCH1 cause more than half of T-ALL cases. NOTCH1 is a transmembrane cell surface receptor that regulates normal T cell development. Upon interaction of the extracellular domain with its respective ligands, an intramembrane presenilin–y-secretase protease complex is activated that liberates the intracellular domain of NOTCH1 - ICN1 - from the lipid bilayer. Then, ICN1 translocates to the nucleus, associates with the transcription factor CSL6 and activates the transcription of a set of target genes including the proliferation promoting oncogene MYC.

Once NOTCH1 receptor is activated by y-secretase complex, y-secretase inhibitors (GSIs) are promising for the treatment of patients with NOTCH1 mutations. There are several GSIs currently under development, one of them, MK-0752 has shown significant gastrointestinal toxicity obtaining only transitory responses, however other inhibitors are being developed and showing moderate efficacy (73).

Combining these inhibitors with glucocorticoids can eventually provide a more effective and safer approach. Studies in cell lines and patient samples demonstrated that combining GSIs and glucocorticoids can induce apoptotic cell death in glucocorticoid resistant T-ALL cells while simultaneously decreasing the gastrointestinal toxicity seen in experimental mice and rats.

The effects of long-term inhibition of y-secretase are unknown; this enzyme targets more than 30 physiologically important transmembrane proteins, including the amyloid precursor protein involved in Alzheimer's disease. There are at least six different y-secretase complexes in humans and subtype specific inhibitors might be developed that have less side effects.

Despite the promise of this approach for NOTCH1-activated T-ALL, not all people with this condition would be expected to respond.

Eight percent of T-ALL samples have mutations or even homozygous deletion of FBXW7, a gene that encodes a ubiquitin ligase that is responsible for NOTCH1 ICN1 turnover. These cells are resistant to treatment with GSIs.

Some cases of T-ALL are suitable to therapies with TKIs. The NUP214-ABL1 rearrangement is present in 5% of T ALL and may benefit of tyrosine kinase inhibitors directed at ABL1. For T-ALL with JAK3 or IL7-R activating mutations there is also the possibility of using TKIs directed to JAK-STAT like tofacitinib (74).

Other targeted molecules are also being studied for T-ALL but one that shows efficacy for all cases of this condition is not yet in the horizon.

6. Conclusion

The advances in standard chemotherapy regimens have reached a plateau, which is motivating the search for other solutions for ALL patients. New pharmacological agents have in common the fact of being directed to specific targets and they can involve the immune system in a more direct or indirect manner.

As these agents are being incorporated in therapeutic regimens several important questions are arising: Can multiple directed agents be incorporated in a single regimen? Should they be used simultaneously or sequentially and what is the optimal sequence? What is the optimal standard chemotherapy dose when using these agents? Can we anticipate the use of conventional chemotherapy for refractory/ relapsed disease in the future? Can we expect much better results for adult patients treated with directed therapies?

The advances in the general support of patients with hematologic cancers like in the treatment of infections, control of cytopenias, therapeutic toxicity management, psychosocial support and the advances in transplant methods are also very important

to consider. They have already contributed a lot for these patient's prognosis and it's necessary to continue investing in this topic.

Waves of new pharmacological agents are passing with many molecules continuously appearing and disappearing from clinical trials. It is an important challenge enrolling patients in the best trial for them.

The price of these new medications is, in general, still very high and it is necessary to study its implementation in our national health care systems and also to discriminate which patients have the best benefit.

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